Analysis of Human Spleen Contamination

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Introduction

Besides carbon, oxygen and nitrogen, numerous other elements and their compounds are significant in the body of humans and other animals. Some elements get into the internal environment of living bodies, accumulate in various organs and disturb systems that participate in their elimination; or, on the contrary, they accumulate in the bodies because of congenital or acquired metabolic disturbances. Iron can be found in human body mainly in the form of ferritin. This protein creates spherical formation with the size of 12 nm. The core of ferritin is 8 nm big and consists of ferrihydride - $5Fe_2O_3.9H2O$. The role of polypeptide coat of ferritin (Ft-H form) is to catalyze Fe^{2+} ions to Fe^{3+} ions. Ft-H form of polypeptide coat helps to mineralization of iron. Studies performed via nanodiffraction showed that physiological ferritin is composed from crystalline ferrihydride and amorphous iron hydroxide [1]. In the core of pathological ferritin prevailed wüstite (FeO) and magnetite – like structure. In excessive volume of iron in the organism, the iron is stored in cells in the form of hemosiderin [2]. Hemosiderin is considered to be a proteolytic product of ferritin [3]. Besides the differences of diffraction image between the samples of hemosiderin it is found also some differences in their element composition [4,5]. The ^{57}Fe Mössbauer spectroscopy enables to determine the composition of iron compound in the sample. Position, number and intensity of absorbed radiation give us information about chemical composition of the sample. Mössbauer spectra and their temperature dependence are characteristic for each iron compound. Biomimetic approach to material synthesis exhibits novel properties. Magnetic materials attract considerable interest both from biological point of view as well as their technological application [6].

Results and Discussion

In the specimens from the adult human spleen, the EDX microanalysis showed the presence of silicon in macrophages of the red pulp (Fig. 1,3). Evaluation of the same specimens in polarized light microscopy did it not uncover. The particles in the spleen were 10-30 µm large. Crystals of SiO2 1-5 µm large are considered to be pathogenic. Shalaeva et al. [7] demonstrated that from the point of silicosis there is no substantial difference between amorphous and crystalline SiO2. Our measurements at another occasion with the help of powder diffraction indicated both, its crystalline and amorphous phase (Fig. 2,4). Our specimens contained silicon, from the viewpoint of silicosis pathogenesis, of inert nature and people whose spleens were investigated, did not show manifestations of silicosis. That is why we suspected that the silicon could have got incorporated into the samples during processing. For this reason we used spleen specimens of dead newborns as controls. There was no silicon present in those specimens. Thus we assume that the silicon must have got into the organism from the external environment. As a foreign material, it was excluded from the blood stream by the spleen clearance function [8]. Different sizes of silicon particles in different parts of the organism were described in electron microscopy with EDAX analysis by Kodaka et al.[9].

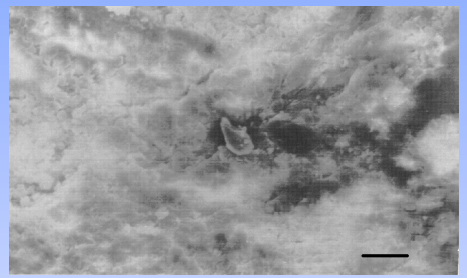


Fig. 1 The particle contains sulphur, aluminum, silicon, chlorium, potassium, calcium and copper. SEM micrograph, line size is 10 µm.

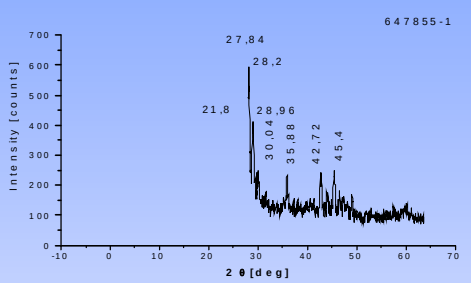


Fig. 2 XRD pattern taken from spleen contains aluminium, silicon, sulphur, chlorine, potassium, calcium. The origin of diffraction peaks is unclear.

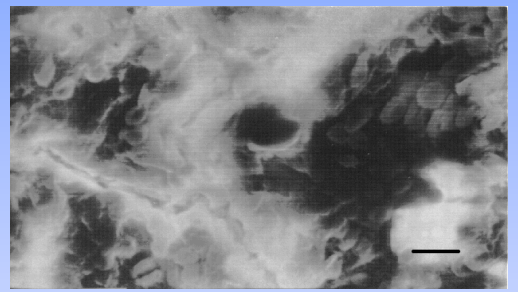


Fig. 3 The particles contain silicon, sulphur, chlorium, potassium, calcium, iron and copper. SEM micrograph, line size is 10 µm.

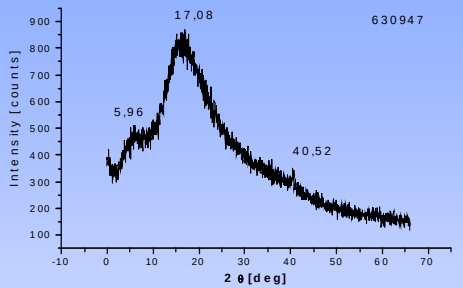
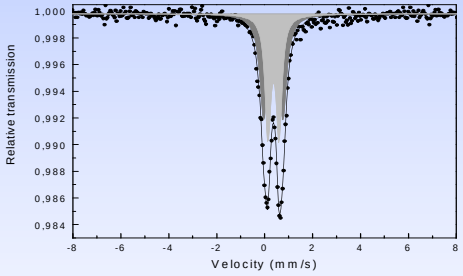
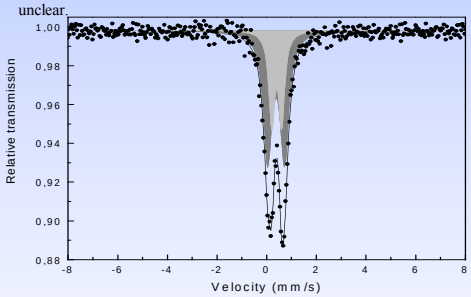


Fig. 4 XRD pattern taken from spleen contains silicon, phosphorus, sulphur, chlorine, potassium, calcium and iron.



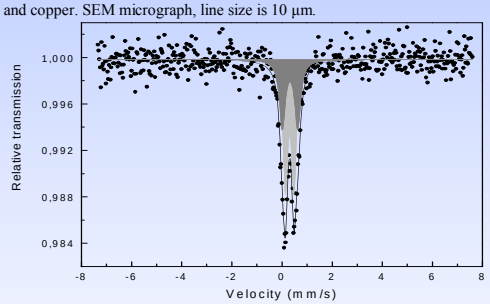
Component	Isomer shift [mm/s]	Quadrupole splitting [mm/s]	Fraction [%]
1	0.36	0.8	50
2	0.37	0.46	50

Tab. 1 Results of isomer shift and quadrupole splitting of spleen tissue after splenectomy with no signs of spleen disease. EDX analysis reveals spectral lines of Mg, Al, Si, P, S, Ca, K, Zn and Fe.



Component	Isomer shift [mm/s]	Quadrupole splitting [mm/s]	Fraction [%]
1	0.37	0.68	67
2	0.4	0.4	33

Tab. 2 Results of isomer shift and quadrupole splitting of spleen tissue from hemochromatosis patient. EDX analysis reveals spectral lines of Mg, Al, P, Si, S, Ca, K, Zn and Fe.



Component	Isomer shift [mm/s]	Quadrupole splitting [mm/s]	Fraction [%]
1	0.32	0.60	40
2	0.31	0.34	60

Tab. 3 Results of isomer shift and quadrupole splitting of spleen tissue with diagnosis of hereditary spherocytosis. EDX analysis reveals spectral lines of Mg, P, Ca and Fe.

Phase of iron oxide	Isomer shift [mm/s]	Quadrupole splitting [mm/s]
Lepidocrocite g-FeO(OH)	0.37	0.53
Feroxyhyte d-FeO(OH)	0.36	0.69
Ferrihydrite $5\ Fe_2O_3 \cdot 9\ H_20$	0.35	0.71
$FePO_4$	0.38	0.80

List of possible phases of the iron oxides present in the studied tissues.

Discussion

The investigation of Perls' Prussian Blue stained slides by light microscope indicates iron depositions in samples with diagnosis of hemochromatosis and hereditary spherocytosis. EDX microanalysis reveals multielemental composition. The core in normal tissue is ferrihydrite - $5Fe_2O_3.9H2O$ with various amount of phosphorus. Sample of spleen tissue with no signs of spleen disease can contain ferrihydrite (Tab. 1, component 1). Presence of vestigial amounts of some elements significantly influences oxidative-reduction status of iron, its structure, chemical composition and stoichiometry [10,11]. Phosphates influence extensively the morphology of iron oxides, if their precursor is ferrihydrite. The amount of phosphorus in iron oxides influences iron oxidation [12]. The interaction of iron Fe(II) with ferrihydrite leads to precipitation of goethite, lepidocrocite and magnetite [13, 14]. The magnetite and goethite-like form of hemosiderin that has been observed in some pathological tissues [15] was not detected (Tab. 2,3). The significant source of Fe(II) is the perturbation in erythrophagocytosis, which plays role in several diseases, including hemochromatosis [16]. We suppose that pH and time are significant factors influence biomineralization of iron in the human spleen.

Literature

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